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REMARKS

It is respectfully requested that this application be reconsidered in view of the above amendments and the following remarks and that all of the claims remaining be allowed.

Claim Numbering

The Communication states that the original copy of the claims submitted by Applicants on September 19, 2003 does not contain claim 25, and claims 21-24 and 26-32 (as submitted on September 19, 2003) have been renumbered as claims 21-31.

Applicants wish to thank the Examiner for confirming by telephone with the undersigned that the original copy of the claims submitted by Applicants on September 19, 2003 does not contain claim 25. The omitted claim 25 has now been added as new claim 32.

Applicants have previously submitted on March 8, 2004 an Amendment in Reply to Office Action in response to the Office Action mailed December 8, 2003 ("the Office Action"), using the original claim numbers. In view of the claim renumbering, the arguments contained in the Amendment in Reply to Office Action submitted on March 8, 2004 are reproduced[1] herein with the current claim numbers for the convenience of the Examiner. This response thus **supersedes** the Amendment in Reply to Office Action submitted on March 8, 2004.

Claim Amendments

Claims 22-24 have been amended to delete the reference to "IL-B50" in the preambles. Since the recitation "IL-B50" in the preamble does not limit the scope of each of the claims, which is fully delineated by the claim body in each case, no new matter has been added by these amendments. The Examiner is hereby requested to enter these amendments.

New claim 32 has been added, for which support can be found, for example, at page 37, line 29 to page 38, line 1.

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[1] In addition, the specification has also been amended to capitalize "FACScan" as "FACSCAN".

Applicants submit that all claim amendments presented herein or previously are made solely in the interest of expediting allowance of the claims and should not be interpreted as acquiescence to any rejections or ground of unpatentability. Applicants reserve the right to file at least one continuing application to pursue any subject matter that is canceled or removed from prosecution due to the amendments.

Election/Restriction (Paragraphs 1-4 of the Office Action)

The Office Action states that the renumbered claims 21-24 and 30-31 have been constructively elected, and claims 25-29 have been withdrawn. Since new claim 32 is also directed to the elected invention, Applicants submit that claims 21-24 and 30-32 are under examination.

Specification (Paragraph 5 of the Office Action)

The Office Action notes the use of a trademark at page 62, line 20, and advises that trademarks should be capitalized and accompanied by the generic terminology. This trademark, FACSCAN®, has been capitalized on pages 57, 58, and 62. It is accompanied by the generic terminology (flow cytometer). Therefore, withdrawal of this objection is respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph (Paragraph 6 of the Office Action)

The rejection of claims 22-24 and 30-31 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention, is respectfully traversed for the reasons set forth below.

Claim 22 is directed to a purified polypeptide wherein the polypeptide comprises SEQ ID NO: 4, or a fragment thereof, capable of binding IL-B50 receptors. Claims 23 and 24 are directed to purified polypeptides comprising an amino acid sequence that is at least about 80% identical to the amino acid sequence of SEQ ID NO: 2 and amino acid residues 1-131 of SEQ ID NO:4, respectively, or a fragment thereof, wherein the polypeptide is capable of binding IL-B50

receptors. Claims 30-31 depend from claims 22-24. The Office Action states that the present application provides SEQ ID NO:2 and SEQ ID NO:4, but allegedly does not provide all proteins that share an 80% identity to SEQ ID NO:2 or amino acid residues 1-131 of SEQ ID NO:4, that are capable of binding IL-B50 receptors.

Pursuant to the Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, Paragraph 1, "Written Description" Requirement, Federal Register 66(4):1099 (2001), possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. *Id.* at 1104.

The present specification describes distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. As the Office Action recognizes, the specification provides the IL-B50 sequences SEQ ID NO:2 and SEQ ID NO:4. The specification further discloses that IL-B50 encompasses a protein having SEQ ID NO:2 or SEQ ID NO:4, significant fragments thereof (page 12, lines 25-28), or proteins or peptides having substantial amino acid sequence identity with the amino acid sequence of the IL-B50 antigen (page 15, lines 7-9). Identity measures will be at least about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90% (page 15, line 29 to page 16, line 2).

The specification also teaches sufficient distinguishing identifying characteristics of the IL-B50 sequences. It is disclosed that the IL-B50 sequence has a signal peptide at the N-terminus, which is about 33 residues long (page 10, lines 23-25). IL-B50 exhibits structural motifs characteristic of a member of the short chain cytokines, such as IL-7 (page 10, lines 26-29). Short chain cytokines are known to have four alpha helices (see, e.g., pages 742-744 of Fundamental Immunology (4^{th} ed.) by Paul, Lippincott-Raven,

Philadelphia-New York; copy enclosed herewith). A sequence alignment of IL-7 and IL-B50, showing four helices, is provided in Figures 1A and 1B. It is further disclosed that helices A and D are most important in receptor interaction (page 40, lines 21-22). Surface exposed residues would affect receptor binding (page 40, lines 25-26). Sequence variants preferably have substitutions away from the conserved cysteines, and often will be in the regions away from the helical structural domains (page 17, lines 7-10). Based on these disclosures, a person of ordinary skill would have agreed that Applicants were in possession of the IL-B50 variants that are encompassed by the rejected claims.

Accordingly, the specification describes sufficient distinguishing identifying characteristics of the claimed invention. Therefore, the written description requirement is satisfied, and withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. §112, Second Paragraph (Paragraphs 7-9 of the Office Action)

The rejection of claims 21-24 and 30-31 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite, is respectfully traversed for the reasons set forth below.

The Office Action states that the term "IL-B50 polypeptide" in claims 21-24 is allegedly indefinite as a limitation. Applicants disagree since the metes and bounds of the claimed polypeptides are clearly delineated by the body of each of the claims, while the preamble term "IL-B50 polypeptide" does not further limit the scope of the claims. Nevertheless, in the interest of expediting prosecution, "IL-B50" has been deleted from the preamble of claims 22-24. Claim 21 does not recite "IL-B50 polypeptide" and is not amended.

Therefore, this rejection is now moot, and its withdrawal is respectfully requested.

Rejection Under 35 U.S.C. §102 (Paragraphs 10-11 of the Office Action)

The rejection of claims 21-24 and 30-31 under 35 U.S.C. §102 in view of Sims et al. (US Patent No. 6,555,520, filed May 9, 2001; hereinafter "the Sims patent") is respectfully traversed for the reasons set forth below.

The Office Action contends that the utility of the claimed IL-B50 sequence is fully disclosed only in the present application, thus taking the position that the effective filing date is established as the filing date of the instant application, namely September 9, 2001. Based on this filing date, the Office Action cites the Sims patent, filed May 9, 2001, as prior art.

It is not required that the utility of a claimed composition to be fully disclosed in a patent application. Pursuant to the Utility Examination Guidelines, Federal Register 66(4), 1092, 1094 (2001): "The patentee is required to disclose only one utility, that is, teach others how to use the invention in at least one way. The patentee is not required to disclose all possible uses". The requirement for utility is that a specific, substantial and credible utility is disclosed for the claimed invention. Federal Register 66(4), at 1098. Credibility of an asserted specific and substantial utility can be assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record (e.g., test data, affidavits or declarations from experts in the art, patents or printed publications) that is probative of the applicant's assertions. Id.

At least one specific, substantial and credible utility has been disclosed in the priority applications for the instant application. For example, U.S. Application No. 60/101,318 (hereinafter "the '318 application"), filed September 21, 1998, discloses that IL-B50 has stimulatory or inhibitory effects on hematopoietic cells, including, e.g., lymphoid cells, such as T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, and hematopoietic progenitors (page 9, lines 9-13 of the '318 application). The '318 application further discloses that IL-B50 is a short chain cytokine exhibiting sequence similarity to IL-7 (page 12, lines 1-4), that IL-B50 and IL-7 are likely to share similar biological functions (page 12, second paragraph, particularly line 32), and that IL-7 exhibits strong effects on lymphopoietic development and differentiation (page 59, lines 21-22). IL-B50 can be used to isolate its receptor (page 49, lines 5-6 of the '318 application), and it was predicted that IL-B50 would bind to the alpha subunit of the IL-7 receptor along with another subunit (page 49, lines 25-28 of the '318 application). Therefore, a skilled artisan would have understood from the '318 application that IL-B50 has similar functions as IL-7, particularly as a stimulating factor for lymphopoietic development and

differentiation. In fact, IL-B50 and IL-7 are so closely related that their receptors would share a common subunit.

These functions are further supported by the data disclosed in the present application. For example, it is shown that IL-B50 induces phosphorylation of Stat3 and Stat5 (page 64, lines 13-16), as well as enhances maturation of dendritic cells (page 67) and expansion/ development of T cells (page 68). The IL-B50 receptor has been identified, and it indeed contains the alpha subunit of the IL-7 receptor (pages 63-64). The data thus indicate that IL-B50 is a hematopoietic cytokine most closely related to IL-7 (page 68, lines 25-26 of the present application) which stimulates lymphopoietic development and differentiation. Thus, the utilities asserted in the '318 application are specific, substantial and credible.

Accordingly, the claimed invention is entitled to the benefit of the filing date of the '318 application, namely September 21, 1998. Since the Sims patent was filed on May 9, 2001, with an earliest possible priority date of November 13, 1998, it is not prior art with respect to the claimed invention. Therefore, withdrawal of this rejection is respectfully requested.

Conclusions

For the reasons set forth above, Applicants submit that the claims of this application are patentable. Reconsideration and withdrawal of the Examiner's objections and rejections are hereby requested. Allowance of the claims remaining in this application is earnestly solicited.

In the event that a telephone conversation could expedite the prosecution of this application, the Examiner is requested to call the undersigned at (650) 839-5044.

Please apply any charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: May 25, 2004

Ping F. Hwung
Reg. No. 44,164

Fish & Richardson P.C.
500 Arguello Street, Suite 500
Redwood City, California 94063
Telephone: (650) 839-5070
Facsimile: (650) 839-5071

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Editor

WILLIAM E. PAUL, M.D.

Laboratory of Immunology
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland

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cell surface receptors. Based on this operational type of definition, it is clear that the distinction between cytokines, growth factors, and hormones often may be imprecise. As a generalization, cytokines and growth factors can be thought of quite similarly, except that molecules involved in host defense that act on white blood cells (leukocytes) are generally called cytokines, whereas those that act on other somatic cell types are more typically described as growth factors. However, there is a major difference between cytokines and hormones. Cytokines generally act locally. For example, in the interaction between a T cell and an antigen-presenting cell, cytokines are produced and usually exert potent actions only locally, and have rather limited half-lives in the circulation. In contrast, after their release, hormones are generally disseminated by the bloodstream throughout the body, acting on a wide range of distal target organs.

In the immune system, terms such as "lymphokines" and "monokines" originally were used to identify the cellular source for the cytokine (1). Thus, interleukin-1 (IL-1), which was first recognized to be made by monocytes, was a monokine, whereas IL-2, which was first described as a T-cell growth factor, was a lymphokine. A major limitation of this nomenclature became evident when it was recognized that many of these lymphokines and monokines were in fact produced by a wide spectrum of cell types, resulting in the adoption of the term "cytokine," first coined by Stanley Cohen in 1974 (2,3). The term, in effect, refers to a factor made by a cell ("cyto") that acts on target cells. The range of actions of cytokines are diverse, including the abilities to induce growth, differentiation, cytolytic activity, apoptosis, and chemotaxis. The term "interleukin" refers to cytokines that are produced by one leukocyte and act on another leukocyte (4). In many cases, however, some interleukins (e.g., IL-1 and IL-6) are additionally produced by other cell types and can act on other cell types, and IL-7 is produced by stromal cells rather than by typical leukocytes.

Among the many different cytokines, the type I cytokines share a similar four α-helical structure, as detailed below, and correspondingly, their receptors also share characteristic features that have led to their description as the cytokine receptor superfamily, or type I cytokine receptors (5–8). Although many of the interleukins are type I cytokines, not all are. For example, of the proinflammatory cytokines IL-1, tumor necrosis factor (TNF)-α, and IL-6, IL-6 is a type I cytokine, whereas IL-1 and TNF-α are not (IL-1 and TNF-α are discussed in Chapter 22). One interleukin, IL-8, is a chemokine (see Chapter 22). Thus, the term "interleukin" refers to a relationship to leukocytes; in contrast, the characterization of a cytokine as a type I cytokine not only has implications regarding its three-dimensional structure, but also has implications related to the structure of its receptor and mechanisms of signal transduction.

In addition to molecules that primarily are of immunologic interest, other extremely important proteins, including growth hormone, prolactin, erythropoietin, thrombopoietin, and leptin, are also type I cytokines and their receptors are members of the same superfamily. As detailed below, these nonimmunologic cytokines share important signal transduction pathways with the type I cytokines of immunologic interest. Thus, the grouping in this chapter emphasizes evolution and signaling pathways, rather than common functions. Hence, although IL-6 exerts many overlapping actions with IL-1 and TNF-α, these latter molecules will be discussed elsewhere because the signaling pathways they use are very different from those common to type I cytokines and their receptors. This raises the important concept, however, that different end functions can be mediated via more than one type of signaling pathway.

The field of interferon (IFN) research has developed in parallel to the cytokine field. IFNs were first recognized as antiviral agents, and as such have been the source of great excitement both for basic science and for potential clinical uses. Over time, it has become clear that the type I cytokines and IFNs share a number of features that now for the first time result in their being addressed together in one chapter in this text. Correspondingly, it is noteworthy that the International Interferon Society changed its name to the International Society of Interferon and Cytokine Research and that the International Cytokine Society focuses on the IFNs, as well as cytokines, together emphasizing the importance of the common themes of IFNs and cytokines that will be the subject of part of this chapter.

TYPE I CYTOKINES AND RECEPTORS

Type I Cytokines: Structural Considerations

Despite the existence of extremely limited amino acid sequence similarities between different type I cytokines, it is striking that all type I cytokines whose three-dimensional structures have been solved (by nuclear magnetic resonance or x-ray crystallographic methods) have similar structures (5–8). Moreover, type I cytokines whose structures have not yet been solved also appear (based on modeling and comparison with the solved structures) to achieve similar three-dimensional structures (5–8). These cytokines are appropriately described as four α-helical bundle cytokines because their three-dimensional structures contain four α-helices (Fig. 1). The first two and last two of these α-helices are each connected by long-overhand loops. This results in an "up-up-down-down" topologic structure because the first two helices (A and B) can be oriented in an up orientation and the last two helices (C and D) can be oriented in a down orientation, as viewed from the NH_2- to COOH-terminal direction. As shown in Fig. 1, the N and C termini of the cytokines are positioned on the same part of the molecule.

Type I cytokines can be grouped as either short-chain or long-chain four α-helical bundle cytokines, based on their size (8). The

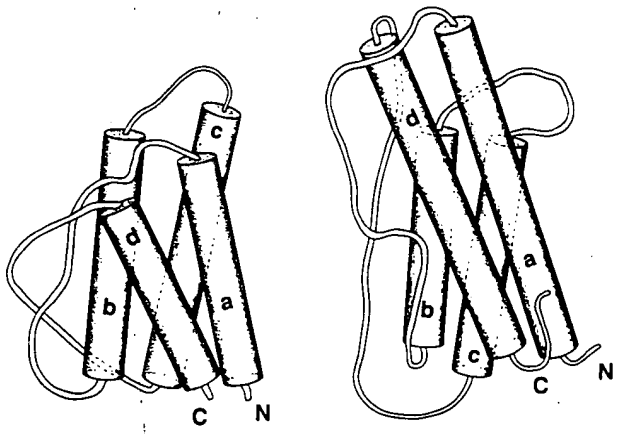

FIG. 1. Four α-helical bundle cytokines. Schematic drawing showing typical short-chain and long-chain four-helical bundle cytokines. Although these both exhibit an "up-up-down-down" topology to their four α helices, note that in the short-chain cytokines, the AB loop is behind the CD loop, whereas in the long-chain cytokines, the situation is reversed. Figure courtesy of Dr. Alex Wlodawer, National Cancer Institute.

short-chain cytokines include IL-2, IL-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-7, IL-9, IL-13, IL-15, monocyte-CSF (M-CSF), and stem cell factor (SCF), whereas the long-chain cytokines include growth hormone, prolactin, erythropoietin, thrombopoietin, leptin, IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), and G-CSF (Table 1) (8,9). In addition to a difference in the length of the helices, which typically are approximately 15 amino acids long for the short-chain helical cytokines and 25 amino acids long for the long-chain helical cytokines, there are differences in the angles between the pairs of helices, and the AB loop is "under" the CD loop in the short cytokines, but "over" the CD loop in the long cytokines (Fig. 1) (7,8,10). Short-chain, but not long-chain, cytokines have β sheet structures in the AB and CD loops. The groupings according to short-chain and long-chain cytokines have evolutionary considerations and also correlate with grouping of receptor chains for these two subfamilies of type I cytokines. An analysis of short-chain cytokines has shown that 61 residues comprise the family framework, including most of the 31 residues that contribute to the buried inner core. The similarities and differences in the structures of IL-2, IL-4, and GM-CSF have been carefully analyzed (6). Among these cytokines, there is considerable variation in the intrachain disulfide bonds that stabilize the structures. For example, IL-4 has three intrachain disulfide bonds, GM-CSF has two, and IL-2 has only one. In IL-4, the first disulfide bond (connecting residues 24 and 65) connects loop AB to BC, the second (connecting residues 46 and 99) connects helix B and loop CD, and the third (connecting residues 3 and 127) connects the residue preceding helix B with helix D. In GM-CSF, the N terminus of helix B and the N terminus of β strand CD are connected by one disulfide bond, whereas the other connects the C terminus of helix C and a strand following helix D. In IL-2, a single essential disulfide bond connects residues 58 and 105 to connect helix B to strand CD. Thus, each cytokine has evolved distinct disulfide bonds to stabilize its structure, although it is typical that helix B is connected to the loop between helices C and D. The structures formed by helices A and D are more rigorously conserved than those formed by helices B and C, primarily due to the interhelical angles; helix D and the connecting region are the most highly conserved elements among the three cytokines (6). This is of particular interest because the regions of type I cytokines that are most important for cytokine–cytokine receptor interactions (based on analogy to the growth hormone receptor structure) include helices A and D and residues in the AB and CD loops, whereas helices B and C do not form direct contacts (6).

Certain variations on these typical structures can occur. For example, IL-5 is unusual in that it is a dimer, positioned in such a fashion so that the ends containing the N and C termini are juxtaposed (11). Helix D is "exchanged" between the two covalently attached molecules so that helix D of each molecule actually forms part of the four-helix bundle of the other monomer (11). M-CSF is also a helical cytokine dimer, but no exchange of helix D occurs (10). The IFNs achieve related albeit somewhat different structures and also are known as type II cytokines (8). IFN-β has an extra helix that is positioned in place of the CD strand (12). IFN-γ is a dimer, each of which consists of six helices (13), as can be seen in Fig. 2. Two of these helices are interchanged, including one from each four-helix bundle (10,13). IL-10, which is closely related to IFN-γ, has a similar structure (14). It is interesting that the majority of helical cytokines have four exons, with helix A in exon 1,

TABLE 1. *Four helical-bundle cytokines*

Short-chain cytokines	Long-chain cytokines
IL-2	IL-6
IL-4	IL-11
IL-7	Oncostatin M
IL-9	Leukemia inhibitory factor
IL-13	CNTF
IL-15	Cardiotropin-1
IL-3	Growth hormone
IL-5[a]	Prolactin
GM-CSF	Erythropoietin (EPO)
	Thrombopoietin (TPO)
M-CSF[a,b]	Leptin
SCF[b]	G-CSF

[a]Dimers.

[b]Different from the other four helical bundle cytokines in the M-CSF and SCF receptors (CSF-1R and c-kit, respectively) have intrinsic tyrosine kinase activity and are not type I cytokine receptors.

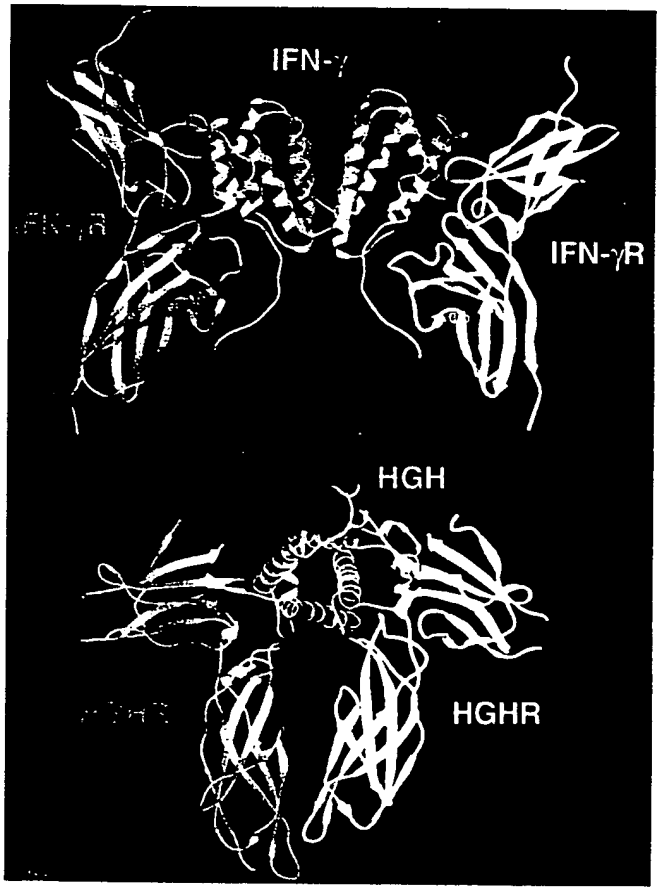

FIG. 2. Structure of the growth hormone and IFN-γ receptors. Shown are ribbon diagrams of the structures of the IFN-γ receptor (*above*) and growth hormone receptor (*below*) as examples of type II and type I cytokine receptors. In the IFN-γ receptor, only IFNGR-1 complexed to the IFN-γ dimer is shown because the full structure with IFNGR-2 is not available. For growth hormone, both growth hormone receptor monomers are shown. The coordinates for the growth hormone–growth hormone receptor structure are from ref. 21 and those for the IFN-γ–IFNGR-1 structure are from ref. 221. Figure courtesy of Dr. Alex Wlodawer, National Cancer Institute.

helices B and C in exon 3, and helix D in exon 4 (7). A related organization is found for IFN-γ, as well as the long-chain helical cytokines growth hormone and G-CSF. However, there are a number exceptions: for example, IL-15 is divided into five exons and IFN-β has only one exon, being devoid of introns (7).

Receptors for Type I Cytokines

The first published report suggesting that type I cytokine receptors had shared features came from a comparison of the sequences of the erythropoietin receptor and the IL-2 receptor β chain (15), but an analysis of a larger number of type I cytokine receptors provided a much clearer view of this new superfamily (16). Type I cytokine receptors are generally type I membrane–spanning glycoproteins (N-terminal extracellular, C-terminal intracellular), the only exceptions being proteins like the CNTF receptor α chain, which lacks a cytoplasmic domain and instead has a glycosylphosphoinositol (GPI) anchor; however, the orientation of this protein is otherwise similar to that of a type I membrane protein. In their extracellular domains, a number of conserved similarities have been noted (Table 2). These include four conserved cysteine residues that were predicted to be involved in intrachain disulfide bonding, and a tryptophan residue, located two amino acids C-terminal from the second conserved cysteine. In addition, a membrane proximal region WSXWS (trp-ser-X-trp-ser) motif was found to be generally conserved, although one exception to this relatively rigorous conservation is found in the growth hormone receptor, in which the motif is a substantially different YGEFS (tyr-gly-glu-phe-ser) sequence.

Interestingly, analysis of a number of the receptors showed that the two sets of cysteines are typically encoded in two adjacent exons, and the exon containing the WSXWS motif is typically just 5′ from the exon encoding the transmembrane domain. Although serines can be encoded by six different codons (i.e., six-fold degeneracy in codon usage), the codons used to encode the serines in WSXWS motif are far more limited, with two of the six possible codons (AGC and AGT) dominating. These data are consistent with a common ancestral precursor. Although many of the known cytokine receptors have been cloned based on expression cloning using a defined ligand, the limited degeneracy of the WSXWS motif has facilitated the complementary DNA (cDNA) cloning of new type I cytokine receptor members (via polymerase chain reaction [PCR]), leading to the first identification of IL-11R (17,18), IL-13Rα (19), and an oncostatin M receptor (20). Another shared feature of type I cytokine receptors is the presence of fibronectin type III domains. In some cases, such as the common cytokine receptor β chain (β_c), which is shared by the IL-3, IL-5, and GM-CSF receptors, the extracellular domain is extended, containing duplications of the domains comprising the four conserved cysteines and the WSXWS motif.

Overall, the different receptor molecules, analogous to the cytokines, have extremely limited sequence identity. Nevertheless, they appear to form similar structures, based on the known structures for the growth hormone prolactin and erythropoietin receptors (21–23), as well as the modeling of other cytokine receptor molecules based on the known structures. Thus, the available data indicate closely related three-dimensional structures for the different type I cytokines and closely related structures for type I cytokine receptors, despite the widely divergent sequences. It is important to note, however, that the only type I cytokine receptors whose structures have been solved correspond to long-chain type I cytokines. The cytokines and their receptors have presumably coevolved, with the differences in amino acid sequences between different cytokines allowing for their distinctive interactions with their cognate receptor chains. At times, however, as illustrated below, despite amino acid differences, a number of sets of cytokines are capable of interacting with shared receptor chains, allowing a number of the different cytokines and their receptors to be grouped into subfamilies (8).

In addition to the above noted similarities in the extracellular domains, there are sequence similarities that are conserved in the cytoplasmic domain of cytokine receptors. In particular, a membrane-proximal region known as the Box 1/Box 2 region is conserved (Table 2), with a proline-rich Box 1 region being the most conserved (24). This will be discussed in greater detail below.

TABLE 2. *Features common to type I cytokine receptors*

Extracellular domain
Four conserved cysteine residues, involved in intrachain disulfide bonds
WSXWS motif
Fibronectin type III modules
Cytoplasmic domain
Box 1/Box 2 regions—the Box 1 region is a proline-rich region that is involved in the interaction of Janus family tyrosine kinases

Type I Cytokine Receptors Are Homodimers, Heterodimers, or Higher Order Receptor Oligomers

The first cytokine receptor structure to be solved was that for growth hormone (Fig. 2) (21). Before the x-ray crystallographic analysis, it was believed that growth hormone bound to its receptor with a stoichiometry of 1:1. Remarkably, however, the x-ray crystal structure solution provided the first evidence that a single growth hormone molecule interacted with a dimer of the growth hormone receptor, in which each receptor monomer contributes a total of seven β strands. Perhaps the most striking finding was that totally different parts of the growth hormone molecule interacted with the same general region of each growth hormone receptor monomer. The three-dimensional x-ray crystal structure for growth hormone and its receptor is shown in Fig. 2. Solving the structure also clarified the basis for growth hormone receptor assembly (21). Kinetically, growth hormone is believed to interact first with one receptor monomer via a relatively large and high-affinity interaction surface (site I), spanning approximately 1,230 Å^2. A second receptor monomer then interacts with the growth hormone–growth hormone receptor complex via two contact points—one on growth hormone (spanning approximately 900 Å^2)(site II), and the other on the first receptor monomer (spanning approximately 500 Å^2) (site III), located much more proximal to the cell membrane. Thus, a total of three extracellular interactions are responsible for the formation of the growth hormone–growth hormone receptor complex. Logically, mutations in site I might prevent receptor binding, whereas mutations in site II would potentially prevent dimerization and signal transduction, providing a rational method for the design of antagonists.

The growth hormone receptor structure showed that the growth hormone receptor extracellular domain is composed of two